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Treatment of Advanced Cancer-related Lymphocytopenia: Comparison among the Effects of Subcutaneous Low-dose Interleukin-2, High-dose Pineal Hormone Melatonin and Checkpoint Inhibitors

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Abstract

Background: Despite the negative prognostic significance of lymphocytopenia is known for many years no clinical cancer study has been proposed up to now in an attempt to specifically correct the evidence of an abnormally low lymphocyte count. At present it is known that IL-2 is the main growth factor for T lymphocytes. Lymphocyte proliferation is also stimulated by the pineal indole hormone melatonin (MLT), which is provided by an anticancer activity, whereas it is inhibited by cortisol.

Objective: On this basis a preliminary study was carried out to evaluate which may be the optimal therapeutic schedule of cancer- related lymphocytopenia.

Methods: The study included 40 advanced solid tumor patients who were treated by the best supportive care (BSC) (n=9), high-dose MLT alone (n=12) at 100 mg/day in the night, subcutaneous low-dose IL-2 alone (n=9) at 1.8 MIU/day for 5 days/week for 2 weeks followed by 2 weeks-rest period, or IL-2 plus MLT. The duration of study was 6 weeks.

Results: The results were compared to those found in 10 advanced cancer patients treated by the checkpoint inhibitor Nivolumab (NVB) at 3 mg/kg/b.w. every 15 days. Lymphocyte count rapidly increased in a significant manner on IL-2 therapy, and a greater increase was reached by Il-2 + MLT. Lymphocyte mean count also increased on MLT alone and on NVB, without, however any statistically significant increase. Finally lymphocyte mean count progressively decreased on BSC alone.

Conclusions: This preliminary study shows that S.C. low-dose IL-2 is the only drug able to rapidly correct cancer-related lymphocytopenia, whose efficacy may be further amplified by the concomitant endocrine therapy with the pineal hormone MLT. Further studies will be required to evaluate the impact of the lymphocytopenia correction on the survival of advanced cancer patients.

Keywords

Checkpoint inhibitors; Immunotherapy; Neuroimmunomodulation; Melatonin; Pineal gland

Introduction

Even though the negative prognostic significance of cancer-related lymphocytopenia in almost all human neoplasms is known for more than 40 years [1] before the same knowledgement of the fundamental role of lymphocytes in mediating the antitumor immunity [2,3], lymphocyte count is not generally taken into consideration by the Oncologists in the clinical management of cancer patients. This behavior should be considered as a clinical limit, since the lymphocyte count may predict not only the prognosis of the neoplastic disease and survival, but also the same efficacy of cancer chemotherapy [4], and of the various antitumor immunotherapies, including the more recent cancer immunotherapy with checkpoint inhibitors, namely anti-PD-1 and anti-CTLA-4 monoclonal antibodies [5,6]. Moreover, in addition to PD1 and CTLA-4 similar results could be obtained with anti PDL1 or PDL2 antibodies. More in detail, the evidence of an abnormal low lymphocyte count prior to chemotherapy has been proven to be associated with a reduced efficacy of chemotherapy itself [7], which may be abrogated by a short-period subcutaneous (SC) low-dose IL-2 before the onset of chemotherapy [8]. On the same way, an evident decline in lymphocyte number on chemotherapy may predict a reduced efficacy of the treatment [7]. On the contrary, an evident lymphocytosis during cancer immunotherapy with IL-2

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has appeared to predict tumor regression or at least a stabilization of the neoplastic diseases [3], whereas the prognostic significance of pre-treatment lymphocyte number and changes in lymphocyte count on immunotherapy with checkpoint inhibitors should be still investigated and defined [5,6]. The negative prognostic significance of lymphocytopenia is independent from the histotype of tumor, since lymphocyte play an essential role in cell-destruction of almost all neoplasms. Cancer-related lymphocytopenia may depend on the same previous anticancer therapies, namely radiotherapy and in a less manner chemotherapy itself, which in some conditions could also enhance lymphocyte count, because of its modulatory effects on cytokine network [4,7], or on the other hand lymphocytopenia may be induced by tumor cells themselves when they express FAS-ligand (FAS-L) molecule, since the interaction between lymphocytes expressing FAS molecule and tumor cells positive for FAS-L may allow the apoptosis of FAS-positive lymphocytes [8]. Some cytokines, such as IL-18, have been shown to stimulate tumor cell FAS-L expression [9]. Moreover, since the antitumor immune response is mainly mediated by lymphocytes, it has to be remarked that cancer-related lymphocytopenia is not a simple epiphenomenon, but it may represent a clinical index provided by both prognostic and physiopathological significance [1-3]. In fact, the evidence of an abnormally low lymphocyte count with values less than 1.000/mm^3, has appeared to be associated with a worse prognosis in most tumor histotypes [1,3,7]. Obviously, the only simple lymphocyte count is not sufficient to establish the immune status of cancer patients, which would require other clinical determinations, consisting of at least the evaluation of the different T lymphocyte subsets, namely T helper-1 (TH1) and regulatory T lymphocytes (T reg) [10,11], since the antitumor immunity is mainly induced by TH1 and inhibited by T reg cells [12,13], as well as the measurement of blood concentrations of the main human antitumor cytokines, represented by IL-2 [2] and IL-12 [14], and the most known pro-tumoral immunosuppressive cytokines, including the pro-inflammatory cytokines, namely TNF-alpha, IL-6, IL-17 and IL-1-beta, and the anti-inflammatory ones, such as TGF-beta and IL-10 [15]. However, from a clinical point of view, the evidence of cancer-related lymphocytopenia has been proven to be mainly due to a decline in TH1 lymphocytes in association with a concomitant increase in T reg cell count and activity [11]. Then, the simple lymphocyte count could reflect at least in part the status of the whole immune functions, including the antitumor immunity. More defined clinical informations may be achieved by detecting the simple lymphocyte-to monocyte ratio (LMR), since a progressive decline in LMR has appeared to predict a poor prognosis in all solid tumor histotypes [16]. This evidence is not surprising, since the antitumor immunity is mainly induced by T lymphocytes and inhibited by the monocyte-macrophage system [17], despite the different functions of the various lymphocyte and monocyte subsets. At present, it is known that lymphocyte proliferation and differentiation may be influenced by both cytokines and immunomodulating neurohormones or neuropeptides, namely the pineal hormones [18,19], and mu-opiod agonists [20]. Within the cytokine group, IL-2 constitutes up to now the only cytokine able to induce a clinically evident increase in lymphocyte count [2,3], namely in TH1 cell number, by representing the main T cell growth factor [2]. Moreover, as far as the neuroimmunomodulation of the antitumor immunity is concerned, at present it is known that the anticancer immune response is mainly stimulated by the pineal hormones, the most investigated of them is melatonin (MLT) [18,19] and inhibited by the mu-opioid agonists [20]. In fact, it has been shown that T lymphocytes may express both MLT and opioid receptors [18] and at present, despite the complexity of the neuroimmunomodulation (NIM), it has been well demonstrated that MLT may activate the antitumor immunity by directly stimulating TH1 cells and playing a major inhibitory effect on T reg cell activity [19], by acting on specific MLT-receptor expressed by lymphocytes themselves, whereas mu-opioid agents may suppress the antitumor immunity by inhibiting TH1-dependent IL-2 secretion and inducing T reg cell generation and activation [21]. On these bases, a preliminary clinical study was planned to establish which may be the most simple, effective, non-toxic and inexpensive strategy to correct cancer-related lymphocytopenia, in an attempt to improve the prognosis of the neoplastic disease, which in contrast is worsened by the evidence of an abnormal low lymphocyte count [1] and to enhance the efficacy of the same antitumor conventional treatments.

Materials and Methods

The study included 40 lymphocytopenic metastatic cancer patients (non-small cell lung cancer n=14, colorectal cancer n=13, gastric cancer n=6, pancreatic adenocarcinoma n= 7), for whom no other effective standard anticancer therapy was available, because of lack of response to previous antitumor treatments, or poor clinical conditions unable to substain a chemotherapeutic approach, then suitable for the only supportive care. Lymphocytopenia was defined as lymphocyte count less than 1,000/mm^3. Eligibility criteria were, as follows: histologically proven solid tumor, metastatic disease, measurable lesions, progression on previous antitumor treatments, life expectancy less than 1 year, and persistent lymphocytopenia, with lymphocyte count less than 1000/mm^3 for at least 3 consecutive months. Because of its immunosuppressive effect on lymphocyte proliferation, patients under chronic therapy with corticosteroids were not included in the study. The experimental protocol was explained to each patient, and written consent was obtained. Patients were randomly treated with the only best supportive care (BSC), with high-dose MLT alone, with subcutaneous (SC) low-dose IL-2, or with IL-2 plus MLT. Moreover, the results were compared to those observed in a group of 10 consecutive metastatic cancer patients (lung cancer: 7; melanoma:3) with lymphocytopenia prior to therapy, who were treated by Nivolumab (NVM), a fully human IgG4 anti-PD-1 monoclonal antibody. Lymphocyte count was considered to be within the normal range when it was greater than 1.500/mm^3 (95% confidence limits). MLT was orally administered at a dose of 100 mg/day once/day during the dark period of the day according to its circadian secretion, generally 30 minutes prior to sleeping. IL-2 was SC injected at a dose of 1.8 M IU/day in the afternoon for 5 consecutive days /week for 2 consecutive weeks, corresponding to one complete IL-2 cycle, by repeating a second cycle after 2 week-rest period. The period of IL-2 injection was limited to only 2 consecutive weeks on the basis of previous clinical studies [8], which have shown no evident further increase in lymphocyte count after 2 weeks of IL-2 therapy. Moreover, according to previous studies, in the group of patients who received IL-2 plus MLT, IL-2 injection was preceded by MLT therapy alone for at least 1 week prior to IL-2 administration, in an attempt to make the immune system of patients more responsive to IL-2 itself. Finally, NVM was intravenously (I.V) injected at a dose of 3 mg/kg b.w. at 15-day intervals for at least 3 consecutive cycles. Data were reported as mean +/- SE, and statistically analyzed by the Student's t test, the chi-square test, and the analysis of variance, as appropriate.

Results

Lymphocyte mean values observed on study are illustrated in Figure 1. Lymphocyte mean number decreased and increased on BSC alone and on high-dose MLT alone, respectively, without, however, statistically significant differences with respect to the pre-treatment values. Moreover, no spontaneous normalization of lymphocyte count was observed on study. On the contrary, a normalization of lymphocyte count, with values more than 1,500/mm^3 was achieved in 2/10 (20%) patients treated by high-dose MLT alone. Lymphocyte mean values significantly increased on SC low-dose IL-2 administration within few days of treatment, and they persisted significantly higher with respect to the number seen prior to therapy during the whole period of the clinical observation ($P<0.01$ *vs* before), and they became within the normal range in 6/10 (60%) patients. The maximal lymphocytosis, however, was obtained by SC low-dose IL-2 plus high-dose MLT, and lymphocyte mean values observed under IL-2 plus MLT were significantly higher with respect to both pre-treatment values ($P<0.001$) and those observed on IL-2 alone ($P<0.05$), with a normalization of lymphocyte count on study in 8/10 (80%) patients. No side-effect occurred on SC low-dose IL-2, and in particular no cardiovascular, renal and neurological toxicity was seen. On the same

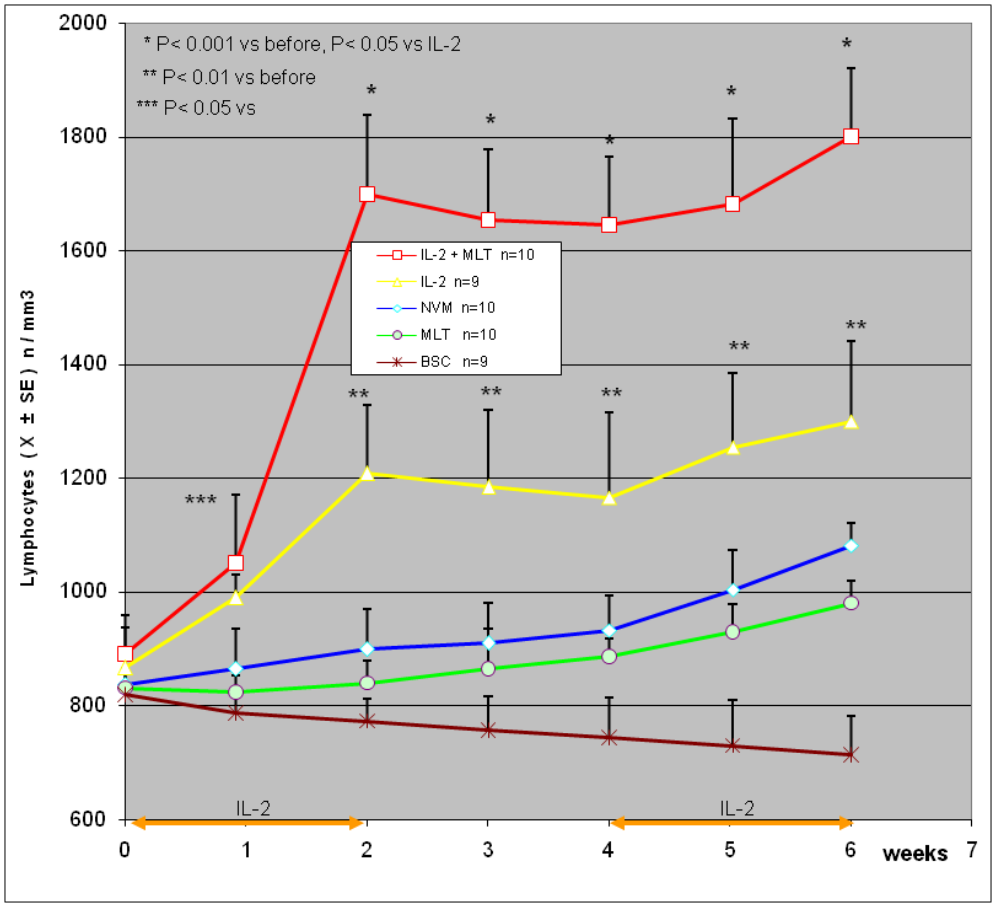

Figure 1: Change in lymphocyte mean count during therapy with best supportive care (BSC) alone, melatonin (MLT) alone, interleukin-2 (IL-2) alone, IL-2 plus MLT or Nivolumab (NVB).

way neither neutropenia, nor thrombocytopenia occurred. Low-grade asthenia was referred by 7/10 (70%) patients treated by IL-2 alone, and in only 2/10 (20%) patients concomitantly treated with MLT. This difference was statistically significant (P<0.05). As far as the immunomodulating effects of NVM are concerned, lymphocyte mean count increased on therapy, without however statistically significant differences with respect to the values observed prior to therapy. Nevertheless, by considering lymphocyte changes in relation to the clinical response to the treatment, patients with partial tumor regression (n=2) or disease stabilization (n=4) showed a statistically significant increase on therapy with respect to the values prior to treatment (1,275 +/- 87 *vs* 842 +/- 68/mm^3, P<0.05), whereas no change was observed in patients who had a progressive disease (n=4) (893 +/- 86 vs 868 +/- 75/mm^3).

Discussion

As expected, since it represents the main or at least apparently the only clinically active lymphocyte growth factor able to clinically increase lymphocyte count, this study shows that IL-2 is the most effective treatment of cancer-related lymphocytopenia, even at non-toxic SC low doses. Moreover, the stimulatory effect of IL-2 on lymphocyte proliferation has appeared to be further amplified by the concomitant administration of pharmacological doses of MLT, and also this evidence is not surprising, since cancer progression has been proven to be constantly associated with a progressive decline in the nocturnal production of the pineal indole MLT, and most in general in the endocrine function of the pineal gland, which constitutes the main anti-tumor organ in the human body, whose diminished function has appeared to be associated with a reduced immunobiological activity of lL-2 itself [8,19]. A clear lymphocyte increase has been observed also in patients under immunotherapy with checkpoint inhibitors, which, however, was limited to the only patients, who achieved a tumor regression, or at least a disease control, by further confirming the fundamental role of lymphocytes in mediating tumor regression and control. Unfortunately, in the past years IL-2 was clinically used just as a potentially anticancer agent, without taking into consideration the immunological nature of the molecule, it was proposed in the treatment of tumors less responsive to chemotherapy, namely renal cancer and malignant melanoma, or in patients, who failed to respond to chemotherapy. In contrast, according to these preliminary results and the more recent discoveries in the antitumor immunity, IL-2 could be used either alone, or in a more favourable way in association with the other most commonly used anticancer therapies, including chemotherapy, radiotherapy and immunotherapy itself, in an attempt to increase lymphocyte number, or at least to counteract the potential chemotherapy and radiotherapy-induced decline in lymphocyte number, since it has been demonstrated a lower efficacy of most antitumor therapies, namely chemotherapy itself [4], in the presence of lymphocytopenia. Because of the fundamental role of lymphocytes in mediating and realizing an effective anticancer immune response, it is very probable that the correction of cancer-related lymphocytopenia by IL-2, the physiological growth factor for lymphocytes [2], could not represent a simple epiphenomenon, but a clinical event, which may allow a greater efficacy of the different anticancer strategies and approaches. Therefore, since lymphocytopenia has been proven to predict a lower survival in cancer patients, the treatment of both spontaneous and anticancer therapy-induced lymphocytopenia would have to be considered as a fundamental strategy in the medical Oncology for cancer cure, irrespectively of the type of treatment and tumor histotypes, being lymphocytopenia one of the main negative prognostic biological parameters [1]. Obviously, further studies, carried out to evaluate IL-2 effects on the different lymphocyte subsets, will be required to better monitor and define the influence of IL-2 on the whole anticancer

immunity, in particular on T reg lymphocytes, which in contrast may suppress the anticancer immune reaction [12,13], because of the possible stimulatory role of IL-2 not only on T helper and cytotoxic T lymphocytes, which mediate the antitumor immune response, but also on T reg cells themselves, event though this event is possible only in the presence of high concentrations of TGF-beta. In any case, the potential stimulatory role of IL-2 on T reg cell generation could display potential therapeutic effects in the autoimmune diseases, which in contrast are characterized by a decline in the functionless of T reg cell system. In addition, lymphocytopenia has recently appeared to be associated with a worse prognosis also in the case of both myocardial and brain infarction, because of the fundamental role of lymphocytes in tissue damage repairing. Therefore, IL-2 therapy could constitute in a near future a fundamental strategy to modulate the immuno-inflammatory response in the overall human systemic diseases. Finally, it has to be remarked that recent clinical observations have demonstrated that a decline in lymphocyte-to-monocyte ratio (LMR) is associated with a negative prognosis in patients with advanced cancer, because of its association with an increase percentage of T reg cell count. Then, the simple evaluation of LMR could represent an inexpensive synthetic biomarker to evaluate the immunological status of the single cancer patient [16,22].

Conclusion

In conclusion, SC low-dose IL-2 may be considered as the most effective and less toxic therapy of advanced cancer-related lymphocytopenia, whose efficacy may be further amplified by the pineal immunomodulating hormone melatonin. An increase in lymphocyte count may be also obtained by the immunotherapy with checkpoint inhibitors, which however would seem to be limited to the only patients who obtained a control of tumor progression. Then, a pretreatment and/or a concomitant therapy with SC low-dose IL-2 could be successfully associated to checkpoint inhibitors in an attempt to enhance their antitumor immune effect.

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